Neuropsychiatric Disease and Treatment

Dovepress

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REVIEW

Role of apolipoprotein E in neurodegenerative diseases

This article was published in the following Dove Press journal:
Neuropsychiatric Disease and Treatment
16 July 2015
Number of times this article has been viewed

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Abstract: Apolipoprotein E (APOE) is a lipid-transport protein abundantly expressed in most neurons in the central nervous system. APOE-dependent alterations of the endocytic pathway can affect different functions. APOE binds to cell-surface receptors to deliver lipids and to the hydrophobic amyloid-β peptide, regulating amyloid-β aggregations and clearances in the brain. Several APOE isoforms with major structural differences were discovered and shown to influence the brain lipid transport, glucose metabolism, neuronal signaling, neuroinflammation, and mitochondrial function. This review will summarize the updated research progress on APOE functions and its role in Alzheimer's disease, Parkinson's disease, cardiovascular diseases, multiple sclerosis, type 2 diabetes mellitus, Type III hyperlipoproteinemia, vascular dementia, and ischemic stroke. Understanding the mutations in APOE, their structural properties, and their isoforms is important to determine its role in various diseases and to advance the development of therapeutic strategies. Targeting APOE may be a potential approach for diagnosis, risk assessment, prevention, and treatment of various neurodegenerative and cardiovascular diseases in humans.

Keywords: apolipoprotein E, pathogenesis, diseases

Introduction

The apolipoprotein E (APOE) gene is located on chromosome 19 and encodes a glycoprotein that is 299 amino acids long.[1] It is synthesized in various tissues in the body including the liver, brain, and skin and in macrophages.[2] In the blood, APOE protein could interact with lipids, resulting in lipoproteins, including very-low-density lipoproteins (VLDL). Several major APOE isoforms can be distinguished: E2, E3, and E4. Six phenotypes were observed as a result of two single nucleotide polymorphisms (SNPs) at amino acid positions 112 and 158. The amino acid changes could alter the protein charge and stability, inducing distinct physiological functions.

APOE plays multiple roles in the regulation of lipid and lipoprotein levels in the blood. APOE serves as a ligand for members of low-density lipoprotein (LDL) receptor family and is involved in the removal of lipoproteins from the circulation for excretion in the liver. APOE is also involved in the formation of chylomicrons and VLDL and affects the activity of other lipid metabolism-associated proteins and enzymes, such as hepatic lipase and lipoprotein lipase. Emerging study has shown that APOE and APOE isoform functions may extend beyond lipid metabolism to include maintenance of normal brain function.[3] In this review, we discuss the biological functions of human APOE and its role in Alzheimer's disease (AD), Parkinson's disease (PD), cardiovascular diseases (CVD), multiple sclerosis (MS), type 2 diabetes mellitus (T2DM), vascular dementia (VD), and ischemic (occlusive) stroke (IS). Targeting APOE may be a potential approach for diagnosis, risk assessment, prevention, and treatment of diseases in humans.

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Importance of APOE

APOE functions

Peripheral system

APOE is a 299 amino acid plasma glycoprotein associated with LDL, VLDL, and high-density lipoproteins (HDL).[4] Several functions of APOE were identified in the human body. In the plasma, APOE will associate with most lipoproteins. APOE is an integral component of chylomicrons, VLDL, and HDL in the peripheral system (Table 1). It operates as part of an anchoring mechanism that aids the transport of triglyceride, phospholipid, cholesteryl esters, and cholesterol into cells by mediating the binding and internalization of these lipoprotein particles.[132] APOE has a strong affinity for and is the main ligand for members of low-density lipoprotein receptor (LDLR) family, located on liver and other tissues. This super family includes the LDLR, LDLR-related protein 1 (LRP1), VLDL receptor, and APOE receptor 2. APOE interaction with LDLR mediates the removal of APOE-containing lipoproteins and modulates the homeostasis of lipids in the peripheral system.

In humans, there are three major isoforms of APOE, which are associated with lipoproteins in the plasma, and absorption of APOE-containing lipoprotein complexes by LDL receptors through lipid metabolism has important implications in diseases (Figure 1).[5,6] Clinical studies have shown that APOE4 is associated with higher plasma total cholesterol and LDL, followed by APOE3 and APOE2.[7,8] This is largely attributed to APOE4 preferentially binding to VLDL and APOE3 to HDL.[9]

The central nervous system

In the central nervous system (CNS), APOE mainly produced by astrocytes (either pericytes or microglia) or under certain pathological conditions (eg, stressors, injurious agents) by neurons.[3,10–12] This involves the redistribution of lipids among cells of different organs, including the CNS (Figure 1).[10] The human brain contains up to 25% of the body's cholesterol, which is essential for myelin production, function, and integrity. Cholesterol homeostasis is important for normal brain functions, since it is an essential component for axonal growth, synaptic formation, and remodeling events that are crucial for learning and memory.[13,14] Cholesterol in the CNS is regulated independently from that in the peripheral system. Cholesterol dysfunction in the CNS could be associated with aging and the development of certain neurodegenerative diseases. In the CNS, APOE mediates cholesterol neuronal delivery.[15,16] The blood–brain barrier restricts the exchange of lipoproteins and APOE between the CNS and peripheral system. A study showed that injury to the brain resulted in an increase in APOE protein in the brain.[17] More recently, two reports have suggested that brain APOE regulates the clearance of amyloid-β (Aβ), which is a common hallmark of some neurological diseases.[18,19] So far, the mechanisms involving APOE in all of these biological processes have not been completely clarified.

Table 1 Plasma lipoproteins containing APOE

Properties	Chylomicrons	VLDL	LDL	HDL
Major apolipoproteins	APOA-I APOB APOC	APOB APOC-I APOC-II APOC-III APOE	APOB	APOA-I APOA-II
Minor apolipoproteins	APOA-II APOE	APOA-I APOA-II APOD	APOC	APOC-I APOC-II APOC-III APOD APOE

Abbreviations: APO, apolipoprotein; APOE, apolipoprotein E; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

APOE polymorphism and mutations

The *APOE* gene is located on chromosome19q13.2. It contains four exons and three introns (Figure 2A), totaling 3,597 base pairs in a cluster with apolipoprotein C1 and apolipoprotein C2. Several individual SNPs have been identified in the human *APOE* gene. In particular, two SNPs, rs7412 (C/T) and rs429358 (C/T), are responsible for the three major alleles: epsilon-2 (ε2), epsilon-3 (ε3), and epsilon-4 (ε4). Because human cells have two copies of each gene, there are six *APOE* genotypes: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4. They are responsible for three homozygous (ε2/ε2, ε3/ε3, and ε4/ε4) and three heterozygous (ε2/ε3, ε2/ε4, and ε3/ε4) genotypes.[11] The three major protein isoforms, APOE2, APOE3, and APOE4, differ from each other by only one or two amino acids at positions 112 and 158. These differences alter APOE structure and function, respectively (Figure 2C).[5,6]

APOE4 is thought to be derived from E3 by a cysteine-to-arginine (Cys → Arg) substitution at position 112 and is designated as E4 (Cys112 → Arg).[5,6] So far, three forms of APOE2 have been described: E2 (Arg158 → Cys), E2 (Arg145 → Cys), and E2 (Lys146 → Gln).[4] In isoelectric focusing, four different mutations give a band at the E2 position, E2 (Arg158 → Cys), E2 (Lys146 → Gln), E2 (Arg145 Cys), and E2-Christchurch (Arg136 → Ser). APOE2 (Arg158 → Cys) is the most common of the ε4.[5,6] APOE1 has been reported to contain a Cys instead

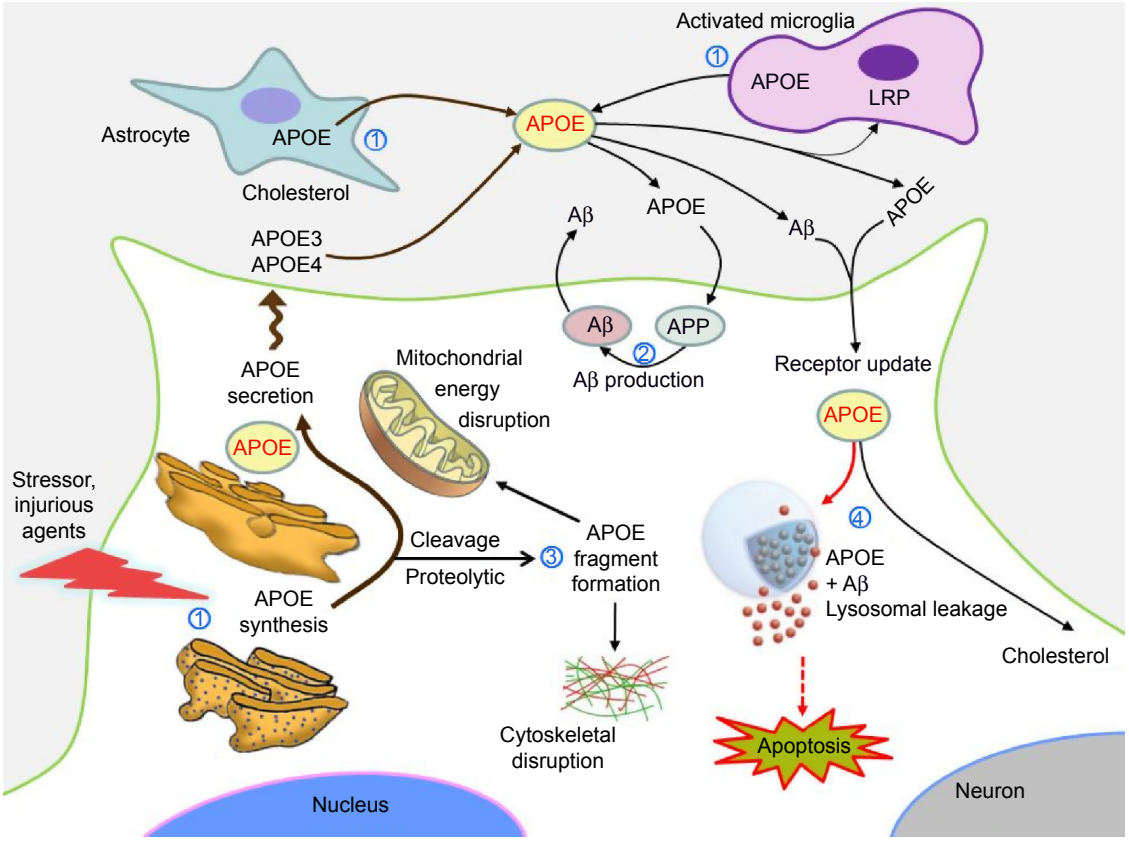

Figure 1 APOE formation and its role in redistribution of lipids to the cells of CNS: the neuropathological effects of the neurotoxic APOE fragments.
Notes: ① APOE mainly produced by astrocytes, either pericytes, microglia, or under certain pathological conditions (stressors, injurious agents, etc). ② The role of APOE in the production of Aβ in association with APP. ③ The result of APOE fragmentation is associated with cytoskeletal disruption and mitochondrial dysfunction. ④ APOE isoform-specifically and Aβ-induced lysosomal leakage and apoptosis.
Abbreviations: Aβ, amyloid-β; APOE, apolipoprotein E; APP, amyloid precursor protein; LRP, lipoprotein receptor-related protein 1; CNS, central nervous system.

of an Arg at position 158, similar to APOE2, as well as an additional amino acid substitution, which probably does not have any functional significance.[20] In addition to these common polymorphisms, several mutations have been described (Table 2). APOE3 is the most-common isoform, while APOE4 and APOE2 are less-frequently observed. The *APOE* ε3 allele is present in 79% the entire population, whereas *APOE* ε4 is only present in 13.3% and *APOE* ε2 in 7.3% of the population.[21] Additionally, there are two rare alleles of the gene, ε1 and ε5, but these are present in <0.1% of the population.[20] The frequencies of the most-common alleles of *APOE* in various populations around the world show that geography, climate, isolation by local adaptations, genetic drift, and possibly evolutionary history selection are responsible for shaping the spectrum of *APOE* genetic variation (Table 2).

APOE

APOE is a 299 amino acid glycoprotein of 34.1 kDa.[1] The structure of this protein varies, depending on the genetic polymorphism. The protein contains two major structural domains, including a compact and stable globular amino-terminal domain (amino acid residues 20–166) and a less-stable carboxy-terminal domain (amino acid residues 225–299).[22] These domains are connected to each other by a hinge region (amino acid residues 166–224).[22] The LDL receptor-binding region is between residues 136–150 of the protein, where multiple basic amino acids are present.[23] The carboxy-terminal domain contains the major lipid-binding region.[24] The amino acid residues 245–266 appear to be critical for binding to VLDL particles, whereas binding to HDL occurs even without the carboxyl-terminal domain.[25] The three major APOE isoforms differ from each other by two cysteine/arginine interchanges at position 112 and 158. APOE2, APOE3, and APOE4 contain cysteine/cysteine, cysteine/arginine, and arginine/arginine at these two positions, respectively (Figure 2C).[26]

The *APOE* ε2 allele carries the Arg158Cys polymorphism. It could disrupt the natural salt bridge between Asp154

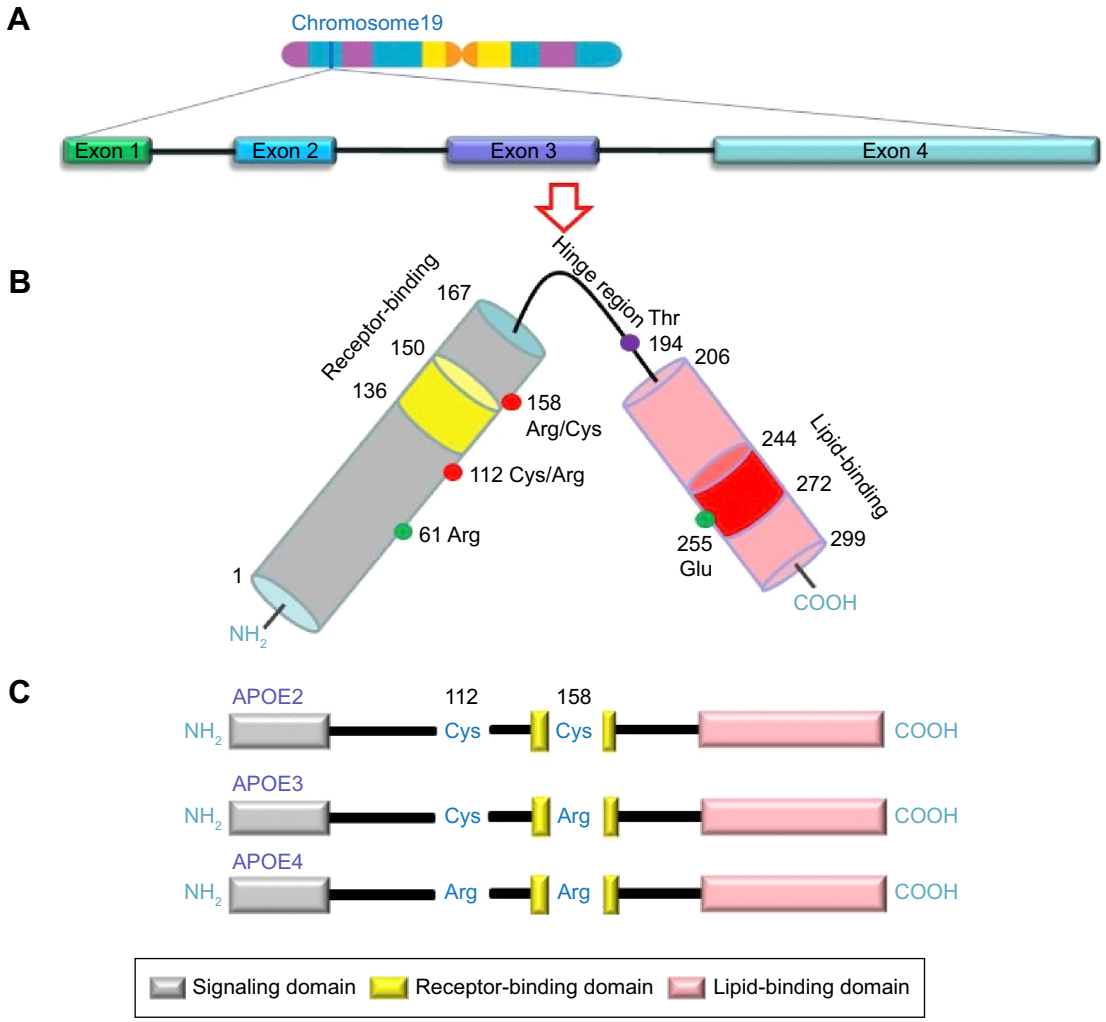

Figure 2 Schematic Illustration of structural and functional regions of APOE.
Notes: (**A**) Location and structure of the APOE gene on chromosome 19. (**B**) APOE protein is a polypeptide chain with 299 amino acids consisting of a receptor-binding region (residues 136–150) in the N-terminal domain (residues 1–167) and a lipid-binding region (residues 244–272) in the C-terminal domain (residues 206–299). (**C**) Three major APOE isoforms are located at residues 112 and 158 (red circles), where APOE2 has Cys residues at both positions, APOE3 has a Cys residue at 112 and an Arg residue at 158, and APOE4 has Arg residues at both positions.
Abbreviation: APOE, apolipoprotein E.

and Arg158. In turn, a salt bridge was formed as the result of the interaction between Arg150 and Asp154. This bridge could disrupt receptor binding because Arg150 is part of the LDL binding site.[27] This mutation was related to type III hyperlipoproteinemia (HLP).[6] Exchange of Asp154 to an alanine could induce the disruption of the Arg150 and Asp154 bridge, and the receptor-binding activity could return to the normal level. Arg150 could relocate within the receptor-binding region.[2] *APOE* ε3 allele presents a cysteine in position 112 and arginine in position 158.[1]

Arg112 mediates two key features of the ε4 allele, as it is associated with reduced stability. The ε4 allele could promote the domain interaction by the orientation of Arg61 on the N-terminal domain. Interaction with Glu255 in the C-terminal domain could be an important structural propriety of the ε4 allele, and ε2 alleles might have less risk for domain interaction. Mutation of Arg61 to Thr (or Glu255 to Ala), was suggested to reduce the domain interaction, causing an ε3-like molecule (Figure 2B).[3] In addition, mutation update and genotype–phenotype correlations of novel and previously described mutations in APOE are available in Table 3.

Measurement of APOE

APOE plays a role in the transport and metabolism of triglyceride-cholesterol. Genotyping could be used to improve the diagnosis of triglyceride cholesterol variants, and APOE polymorphisms were also associated with altered odds of having AD and other diseases. Determination of APOE level is of potential interest when studying different forms of brain damage and as a marker of ongoing regenerative processes in the brain. APOE is a polymorphic apolipoprotein exhibiting three major isoforms, ε2, ε3, and ε4, at a single gene locus.

Table 2 Relative frequencies of the most-common alleles for the gene locus coding for APOE in various populations of the world

Population	n	ε2	ε3	ε4	Reference
European					
Lapp	70	0.050	0.640	0.310	35
Swedish	279	0.119	0.675	0.206	133
Danish	466	0.085	0.741	0.174	134
Finnish	2,245	0.044	0.748	0.208	133
Dutch	2,218	0.085	0.752	0.163	133
Belgian	760	0.072	0.765	0.163	135
Icelandic	185	0.068	0.767	0.165	133
United Kingdom	734	0.089	0.767	0.144	133, 136
French	1,228	0.108	0.771	0.121	133
German	2,031	0.077	0.778	0.145	133
Norwegian	395	0.087	0.781	0.132	133
Tyrolean	469	0.090	0.789	0.117	133
Hungarian	202	0.064	0.807	0.129	133
Swiss	173	0.072	0.821	0.107	133
Polish	137	0.055	0.839	0.106	137
Italian	2,000	0.060	0.849	0.091	133
Spanish	1,286	0.052	0.856	0.091	36, 134, 136, 138
Sardinian	280	0.050	0.898	0.052	133
African					
Zambian	116	0.138	0.598	0.267	139
Brazilian	123	0.610	0.805	0.134	140
Pygmy	70	0.057	0.536	0.047	141
Khoisan	247	0.077	0.553	0.370	142
Moroccan	100	0.065	0.850	0.085	138
Nigerian	365	0.027	0.677	0.296	143
Sub-Saharan	470	0.116	0.706	0.178	141
Beninese	97	0.103	0.742	0.155	144
Ethiopian	164	0.031	0.811	0.143	144
Sudanese	105	0.081	0.619	0.291	143
Asian					
Bangladeshi	53	0.050	0.800	0.150	145
Indian	497	0.051	0.881	0.068	21
Malay Aboriginal	223	0.140	0.620	0.240	146
Malay	118	0.114	0.767	0.119	143
Chinese	1,034	0.105	0.824	0.071	76, 134, 143, 147
Japanese	1,097	0.048	0.851	0.101	143
Korean	305	0.127	0.750	0.121	148
Native American					
Cayapa	91	0.000	0.720	0.280	149
Amerindian	110	0.000	0.816	0.184	150
Yanomami	96	0.000	0.844	0.156	151
Mayan	135	0.000	0.911	0.089	150
Oceanian					
Papuan	110	0.145	0.486	0.368	150
Polynesian	111	0.110	0.630	0.260	150
Aboriginal Australian	64	0.0	0.740	0.260	150

Abbreviation: APOE, apolipoprotein E.

APOE isoform-specific effects on APOE/Aβ complex levels may mediate the increase in soluble Aβ levels that correlate with APOE4. Allelic variations in *APOE* were consistently associated with plasma concentrations of total cholesterol, LDL cholesterol, and APOB (the major protein of LDL, VLDL, and chylomicrons). *APOE* ε2 was studied in disorders associated with elevated cholesterol levels or lipid derangements such as type III HLP, coronary heart disease, stroke, peripheral artery disease, and diabetes mellitus.[8] *APOE* ε2 was established as an important marker for diagnosis. *APOE* ε4 is a major genetic risk factor for neurodegenerative diseases such as AD and PD.[28–30]

Methods have been developed to detect individual *APOE* phenotype or genotype. Studies indicated that the method for detection and the source of the components for APOE/Aβ complex were critical parameters for the experimental outcome. So far, many methods have been developed to measure APOE/Aβ complex, including gel-shift assay on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, Western blot analysis,[31] co-immunoprecipitation,[31] size-exclusion chromatography/gel-filtration, and enzyme-linked immunosorbent assay.[32] Some *APOE* variants that were defective in their ability to mediate the binding of lipoproteins to the LDL receptor and that are associated with diseases were poorly recognized by antibodies. Moreover, numerous studies have been developed to measure the effect of *APOE* polymorphism on APOE/Aβ complex formation using these methods (Table 4).

APOE-associated diseases

Understanding structural differences in APOE isoforms helped establish the molecular mechanism responsible for the associated pathology. Defects in APOE could result in alterations in its structure and function.[33] The critical effect of APOE in regulating plasma lipid and lipoprotein levels has been extensively and carefully studied.[3,13,33–37] Evidence indicates its association with neurodegenerative diseases and also other chronic diseases. This review will summarize the critical available data related to APOE defects and their role in AD, PD, CVD, type III HLP, MS, T2DM, VD, and IS (Figure 3).

Alzheimer's disease

AD was originally described by Alois Alzheimer in 1907.[38] It is the most-common age-related dementing illness, which is currently estimated to affect 35.6 million individuals worldwide.[39] It was estimated that the number of patients with AD will triple by 2050.[40] APOE plays a critical role in transporting cholesterol in and out of the CNS and is also recognized as the most important risk factor for the late-onset form of AD. The distribution of APOE's three major alleles, ε2, ε3, and ε4, in patients with AD is 3.9%, 59.4%, and 36.7%, respectively.[30] Based on the strong association between APOE and Aβ in the brain,[41] APOE was suggested

Table 3 Variants of human APOE

Type of genetic variation	Designation	Disease association	Reference
Point mutation	E1 (Gly127 → Asp, Arg158 → Cys)	HLP	85
	E1 (Lys146 → Glu)	Type III HLP	81
	E1 (Lys146 → Asn; Arg147 → Trp)	Type III HLP	79
	E1 (Arg158 → Cys; Arg180 → Cys)	Hypertriglyceridemia	80
	E1 (Arg158 → Cys; Leu252 → Glu)	Type IIa HLP	152
	E2 (Arg25 → Cys)	Type III HLP	153
	E2 (Arg134 → Glu)		78
	E2 (Arg136 → Ser)	Type III or V HLP	154
	E2 (Arg136 → Cys)	Type III HLP	82, 84
	E2 (Arg142 → Leu)	Type III HLP	155
	E2 (Arg145 → Cys)	Type III HLP	4
	E2 (Arg145 → Prol)	Lipoprotein glomerulopathy	156
	E2 (Lys146 → Gln)	Type III HLP	78, 83
	E2 (Gln187 → Glu)	Type III HLP	157
	E2 (Arg224 → Gln)	Xanthomatosis Hyperlipidemia	158
	E2 (Arg228 → Cys)	Type IV or V HLP	159
	E2 (Val236 → Glu)	Type IIb or IV HLP	152
	E3 (Ala99 → Thr; Ala152 → Prol)	Hyperlipidemia	160, 161
	E3 (Ala106 → Val)	Hypertriglyceridemia Alzheimer's disease	162
	E3 (Cys112 → Arg; Arg142 → Cys)	Type III HLP	1
	E3 (Cys112 → Arg; Arg251 → Gly)	Type IV HLP	152
	E3 (Arg136 → His)	Type III HLP	163
	E3 (Thr42 → Ala)		164
	E3 (Arg145 → His)	Hyperlipidemia	165
	E4 (Glu13 → Lys; Arg145 → Cys)	Type III HLP	135, 166
	E4 (Leu28 → Prol; Cys112 → Arg)m	Alzheimer's disease Coronary artery disease Types IIa, IIb, IV, and V HLP	167, 168
	E4 (Cys112 → Arg; Arg274 → His)		152
	E4 (Ser296 → Arg)		152
	E5 (Glu3 → Lys)	Hypercholesterolemia	169
	E5 (Glu13 → Lys)		170
	E5 (Gln81 → Lys; Cys112 → Arg)	Hypercholesterolemia	171
	E5 (Pro84 → Arg; Cys112 → Arg)		169
	E5 (Glu212 → Lys)		172
	E7 (Glu244 → Lys; Glu245 → Lys)	Hyperlipidemia	173, 174
	E_{Null} (Trp210 → Stop)	Type III HLP	175
	E_{Null} (TGG20 → Stop)	HLP	176
Deletion	E1 (Gln156–Gly173 → 0)	Lipoprotein glomerulopathy Systemic atherosclerosis	177
	E1 (Leu141–Lys143 → 0)	Lipoprotein glomerulopathy	178
	E_{Null} (Leu60 → Stop)	Hyperlipidemia	179
	E_{Null} (Leu229 → Stop)	Type III HLP	180
	APOE protein is abnormally spliced	Type III HLP	181
Insertion	E3 (Cys112 → Arg, duplication 120–126)	Type IV HLP	182
	E5 (duplication 135–142)	Type IV HLP	183

Abbreviations: APOE, apolipoprotein E; HLP, Hyperlipoproteinemia.

as an Aβ-binding protein that induces a pathological β sheet conformational change in Aβ.[42] *APOE* ε4 suggests probably increases the risk of AD by initiating and accelerating Aβ accumulation, aggregation, and deposition in the brain. Cleavage of APOE4 may increase AD risk in two ways, either through a loss of function or gain of toxicity.[30] Genome-wide association studies have shown that the *APOE* ε4 allele is associated with AD,[43–45] and was detected in homogeneous and heterogeneous populations in North America, Europe, and Asia.[46–48]

Table 4 The methods utilized to measure APOE/Aβ levels

Human APOE source	Detection method	Results	Reference
Human plasma (purified)	SDS–PAGE (nonreducing), WB	APOE4/Aβ > APOE3/Aβ Stability at 4.6 pH = APOE3/Aβ > APOE4/Aβ	45
Human plasma (purified)	SDS–PAGE (nonreducing), WB	APOE3/Aβ > APOE4/Aβ	184
Human brain (AD and NAD)	SDS–PAGE	AD > NAD, no APOE isoform differences measured	41
Human plasma (purified)	Surface plasmon resonance	APOE3/Aβ > APOE4/Aβ = APOE2/Aβ	185
CHO (CM) Human plasma	SDS–PAGE (nonreducing), WB	APOE3/Aβ = APOE2/Aβ >> APOE4/Aβ (ND)	186
Human plasma APOE	IP with SDS–PAGE, WB	NAD APOE23/Aβ = NAD APOE33/Aβ = NAD APOE34/Aβ >> AD APOE33/Aβ > AD APOE44/Aβ SDS and protease digestion stability: NAD > AD	31
Recombinant (nonlipidated and lipidated)	ELISA	APOE2/Aβ > APOE3/Aβ > APOE4/Aβ	174
RAW264 and HEK293 (CM, delipidated) Sf9 insect cells (delipidated and lipidated)	ELISA	CM and Sf9 (lipidated): APOE3/Aβ > APOE4/Aβ All sources (delipidated): APOE3/Aβ = APOE4/Aβ	32
RAW264 (CM)	Co-IP, SDS–PAGE (nonreducing), WB	CM: APOE3/Aβ >> APOE4/Aβ	187
CSF (NAD ε3/ε3, PAD ε3/ε4, AD ε4/ε4)		CSF: APOE33/Aβ > APOE34/Aβ = APOE4/Aβ (ND)	
(Lipidated) Recombinant	SDS–PAGE (nonreducing), WB	APOE3/sAβ > APOE3/agg Aβ	188
HEK293 (CM)	(Nonreducing), WB SDS–PAGE	APOE3/oAβ > APOE3/Aβ fibrils > APOE4/oAβ > APOE4/Aβ fibrils	33
(Lipidated) Recombinant	ELISA	Intermediate agg Aβ40: APOE4/Aβ >> APOE2/Aβ = APOE3/Aβ	189
Escherichia coli (purified and lipidated)	EPR spectroscopy	Purified APOE: APOE3/oAβ > APOE4/oAβ Lipidated APOE: APOE3/Aβ > APOE4/Aβ	190
Human plasma (NAD) Human CSF (NAD)	SEC, SDS–PAGE (nonreducing), WB	95% Aβ elutes with lipoproteins 100% Aβ associated with APOE-containing lipoproteins APOE monomer/Aβ (45 kDa) and APOE dimer/Aβ (97 kDa) detected	34
Hippocampal homogenates (EFAD mice)	ELISA	SDS stable: E2FAD > E3FAD > E4FAD Total complex: E2FAD = E3FAD > E4FAD	191
Human cortical synaptosomes (AD and NAD) Human CSF (AD and NAD)		Total complex: NAD > AD NAD APOE33/Aβ = NAD APOE4X/Aβ >> AD APOE33/Aβ > AD APOE4X/Aβ SDS stable: NAD APOE33/Aβ >> NAD APOE4/Aβ NAD > AD, NAD APOE33/Aβ > AD APOE33/Aβ > AD APOE44/Aβ	

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; agg Aβ, aggregated amyloid-β; APOE, apolipoprotein E; CHO, Chinese hamster ovary cell; CM, conditioned media; co-IP, co-immunoprecipitation; CSF, cerebrospinal fluid; EFAD, essential fatty acid deficiency; EPR, electron paramagnetic resonance; ELISA, enzyme-linked immunosorbent assay; IP, immunoprecipitation; NAD, non-Alzheimer's disease or nondementia control; ND, not detectable; oAβ, oligomeric amyloid-β; PAD, probable Alzheimer's disease; PAGE, polyacrylamide gel electrophoresis; sAβ, soluble amyloid-β; Sf9, *Spodoptera frugiperda* insect cells; SDS, sodium dodecyl sulfate; SEC, size-exclusion chromatography; WB, Western blot.

Since the relationship between APOE immunoreactivity and amyloid plaques was first reported,[42,49] the ε4 allele has shown to be strongly associated with both late-onset familial[45,50] and sporadic AD.[43,44] It is estimated to be the principal genetic factor in up to 50% of all cases of AD.[51,52] Moreover, risk for AD was increased two- to threefold in individuals who carried the heterozygous ε4 allele and about 12-fold in those who carried the homozygous ε4 allele.[51,53] Conversely, there was evidence suggesting that the *APOE* ε2 allele may be protective against AD or associated with a marked reduction in AD risk.[54,55] AD risk in the *APOE* ε2/ε2 or ε2/ε3 carriers was lower than in those carrying ε3/ε3.[55] APOE's role in AD is well established. Therefore, further studies are needed to understand the possible association between APOE and the rate of disease progression. APOE was classified as a risk factor for AD, but the molecular events that precede dementia remain elusive. APOE4 was suggested to be associated with damage of the vascular system in the brain, leading to AD pathogenesis.

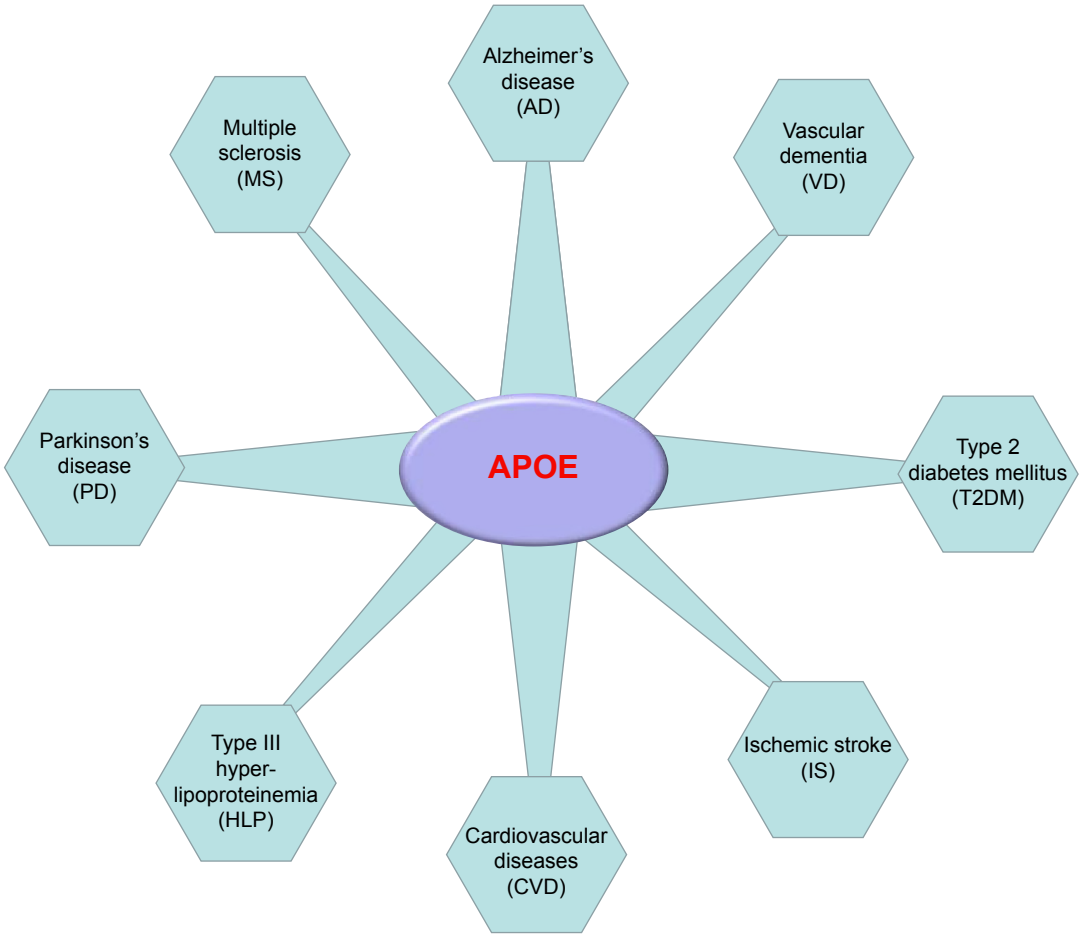

Figure 3 APOE is associated with disease progression in various conditions.
Abbreviation: APOE, apolipoprotein E.

Vascular dementia

VD is a general term describing problems with reasoning, planning, judgment, memory and other thought processes caused by brain damage from impaired blood flow to your brain. The problem with this disease is the lack of consensus on the pathological criteria required for the exact diagnosis. The prevalence of VD in individuals older than 65 years of age is estimated to be 1.2%–4.2%. Moreover, there are an estimated 6–12 cases per 1,000 persons older than 70 years of age per year.[56] It is difficult to distinguish the prevalence between VD and other pathologies, as 20%–30% of demented subjects show mixed pathologies.[57] Clinically, VD and AD share pathological features such as the presence of neurofibrillary tangles, amyloid plaques, white-matter lesions, and cerebral amyloid angiopathy.[58]

There are many VD risk factors, including hypertension, stroke, atherosclerosis, and other metabolic disorders. However, APOE was also considered as an important risk factor for VD. The role of APOE4 in the development of VD might be an area of intensive investigation, with conflicting conclusions. The consumption of highly saturated fat and cholesterol may confer added risk for the development of VD, which would be aggravated even more in people carrying the ε4 allele.[59,60] Some studies have shown a positive association between harboring the ε4 allele and increased risk for VD,[61–64] while others have indicated that ε4 allele might not be associated with VD risks.[65,66] Recent meta-analyses have revealed evidence of an increased VD risk in individuals with *APOE* ε4 compared with *APOE* ε3.[64] Besides, *APOE* ε4 contribution to the risk of vascular cognitive impairment is independent of other vascular risk factors, including hypertension, dyslipidemia, and atherogenesis.[67] The presence of APOE in patients with VD could provide pathological evidence supporting the potential link between *APOE* polymorphism and enhanced risk for VD.

Cardiovascular diseases

CVD, including coronary heart disease, refers to any diseases that affect the cardiovascular system – principally cardiac

disease, vascular diseases of the brain and kidney, and peripheral artery disease.[68] The biological activity of APOE can be influenced by modification of its structure and/or quantity. Epidemiologic studies have indicated the direct association between APOE and CVD as well as its impact on cholesterol levels. In a study in middle-aged men, it was estimated that 40% of the ε4 carrier population had an increased risk for CVD mortality compared with individuals with the ε3/ε3 or ε2 genotypes.[69] Studies indicated that ε4 carriers had an increased risk of death from CVD.[35,70,71] Certain studies have linked the ε4 allele with a greater risk for coronary artery disease and myocardial infarction. Indicatively, higher frequency of ε4 was associated with higher cholesterol levels and higher CVD mortality rates in Finland, Scotland, and Northern Ireland.[71,72] Furthermore, an increased CVD risk was also associated with the ε2 allele.[70] A report on the frequency of *APOE* genotype and its related CVD indicated that American Indians, Asians, and Mexican Americans presented the highest frequency of E3 (>84%). Africans and African Americans presented the highest frequency of E4 (20.1% and 31%, respectively). African Americans and Caucasians (except Finns) presented the highest frequency of E2 (7.3%–13.1%).[8]

So far, several studies have suggested that the *APOE* ε4 allele is a risk allele for CVD, while others failed to find any association. The dual role of APOE remains enigmatic and needs to be further explored in order to elucidate its precise role in cardiovascular and cerebrovascular diseases.

Type III hyperlipoproteinemia

Type III HLP, also known as dysbetalipoproteinemia or broad beta disease, is a genetic disorder characterized by accumulation of remnant lipoproteins in the plasma and development of premature atherosclerosis.[10] Type III HLP results from the accumulation of chylomicron remnants from intestinal lipoproteins and VLDL remnants derived from hepatic lipoproteins. The expression of different isoforms of APOE that do not bind to the receptor[2,10,73] and APOE deficiency was associated with the development of type III HLP.[74,75] The primary molecular cause of type III HLP was also associated with the presence of APOE2.[6] APOE2 increased triglyceride and cholesterol levels, leading to delayed clearance of hepatic and intestinal remnant lipoproteins, resulting in type III HLP.[3] The development of overt hyperlipidemia requires the inheritance of two alleles of *APOE* ε2, and most ε2/ε2 allele carriers are either normolipidemic or even hypocholesterolemic. Some studies showed that type III HLP could occur with a frequency of 1–5 per 5,000 individuals. Meanwhile, in Caucasian populations, type III HLP occurred with a frequency of 0.5–1.0 per 100 individuals with the appearance of ε2/ε2 homozygosity.[73,76] Moreover, more than 90% of patients with type III HLP were homozygous for the ε2/ε2 (Arg158 → Cys) allele, and the disease is normally considered as a recessively inherited multifactorial trait.[77] On the other hand, a variety of rare naturally occurring APOE mutations were also described that are associated with the dominant mode of inheritance of type III HLP at an early age.[78–85]

Parkinson's disease

PD and AD share some clinical and neuropathological features.[86] PD progresses slowly in most people, affecting 2% of the population older than 65 years of age.[29] APOE isoforms might affect degenerative processes by changing the lipid metabolism. In the CNS, the association between APOE and PD has been demonstrated.[87,88] Most studies failed to report any association between *APOE* ε4 and susceptibility to PD and PD-associated dementia.[28,29] Thus, several studies focused on ε4 as a risk factor for age of onset and decrease in cognitive impairment associated with dementia in PD. However, ε2 was considered as a weak or inconsistent risk factor for PD.[29,89–91] One meta-analysis indicated that the ε2 allele was associated with higher risk of PD development,[92,93] whereas another study indicated that the ε4 allele could be responsible for PD development.[87] So far, studies focusing on the role of APOE in PD remain largely inconclusive.

Type 2 diabetes mellitus

T2DM is typically a chronic disease, and its prevalence increases with age. T2DM is also the predominant type, accounting for 90% of diabetes mellitus cases.[94] T2DM has been affecting nearly 4% of the world's population, and it may be increasing up to 5.4% by year 2025.[95] The development of T2DM could be caused by a combination of lifestyle and genetic factors.[96,97] Recently, studies have showed that older patients with T2DM have a higher risk of cognitive dysfunction or dementia.[98] Moreover, T2DM was related not only to VD, but also to the clinical diagnosis of AD-type dementia.[99]

APOE could play an important role in the regulation of plasma and cellular lipid concentrations,[37] and APOE isoforms could present differences in chemical stability.[100] *APOE* polymorphism could be one of the factors that affects the development of T2DM. Some studies demonstrated that *APOE* ε2 allele was associated with an increased risk of T2DM.[101–104] In addition, global statistics show that the large burden of T2DM is restricted to developed countries.

It is also a remarkable problem for developing countries such as People's Republic of China[105] and India.[106] A meta-analysis of 29 studies, which included 4,615 T2DM cases and 2,867 controls in the Chinese Han population, indicated that the *APOE* ε2 and ε4 alleles may be associated with increased risk of T2DM and diabetic nephropathy.[107] In addition, some studies have indicated that *APOE* ε4 associated with the risk of T2DM.[108–110] In fact, deficits in cognitive performance were observed only for those with T2DM and, at least, one *APOE* ε4 allele.[111] The association between T2DM and AD was particularly strong among carriers of the *APOE* ε4 allele. T2DM is associated with reduced cognitive function and the incidence of dementia, including AD. The underlying mechanism of this association should be elucidated.

Multiple sclerosis

MS is the most-common autoimmune disorder affecting the CNS.[112] The disease usually begins between the ages of 20 and 50, and it could be twice as common in women as in men.[113] A genetic linkage between the chromosome 19q13 region and MS has been demonstrated.[114,115] So far, its association with *APOE* genotype remains unclear, and study results have been inconsistent. Some studies suggested that *APOE* ε4 might be a modifier of MS progression, increasing damage to the brain and worsening cognitive dysfunction and disease severity.[116,117] However, these conclusions remained controversial.[118] The possible association between ε4 and cognitive dysfunction in MS has been investigated in a handful of studies with conflicting results.[117,119,120] However, *APOE* ε4 allele has been indicated as a risk factor for cognitive impairment in MS.[116,117,119, 121] Furthermore, patients with MS who carried the ε4 allele were also reported to present verbal memory deficits.[119,122] Recently, a study indicated that both ε2 and ε4 exert relevant effects on MS susceptibility, on the basis of combined data from 13,913 MS cases and 15,831 controls.[123] So far, APOE association studies in MS have mostly yielded negative results, with some studies reporting significant effects and others being unable to confirm these associations.

Ischemic stroke

IS is one of the most frequent causes of mortality and disability worldwide.[124] Approximately 17 million people had a stroke in 2010, and 33 million people have previously had a stroke and were still alive according to the World Health Organization. Moreover, overall two-thirds of strokes occurred in individuals older than 65 years of age.[125] IS shares several risk factors with heart disease and it also occurs when an artery to the brain is blocked. APOE may have an impact on stroke occurrence. The ε4 allele was associated with increased levels of LDL and cholesterol as well as ischemic heart disease.[8,126] Initial studies assessing outcomes after IS demonstrated that *APOE* ε4 was not associated with poor prognosis,[127,128] and a meta-analysis reported a significant association between IS and the ε4 allele.[129] Recently, IS prevalence was demonstrated to be significantly greater in ε4 carrier patients.[130] Furthermore, a meta-analysis of 22 published studies with a total of 30,879 subjects showed that the ε4 allele was related to increased carotid intima-media thickness, a factor associated with IS.[131] IS is a result of complex interactions between environmental and genetic factors. The influence of each gene is expected to be modest. However, it is possible that the tremendous impact of acquired risk factors on stroke occurrence may obscure or eliminate a possible genetic influence on the disease pathophysiology. *APOE* ε4 seems to be the best candidate for studying the interplay between genetic and acquired risk factors.

Conclusion

This review highlighted the association between APOE function and the development of associated diseases. Increasing evidence has suggested a central role for APOE in modulating processes of neurodegeneration, as described. APOE isoforms differentially regulate Aβ aggregation and clearance in the brain and have distinct functions in regulating brain lipid transport, glucose metabolism, neuronal signaling, and neuroinflammation. APOE isoforms probably accelerate the rate of disease conversion and progression. Therefore, studying APOE and its function may enable the identification of disease risks, which may allow an earlier identification of individuals with the disease. APOE genotype status might help predict a clinical diagnosis and assess treatment efficacy using tools such as putative AD biomarkers, magnetic resonance imaging scans, and measurements of Aβ. In addition, APOE isoforms have differential roles in maintaining vascular health, which is crucial because vascular pathology is strongly associated not only with AD, but also with others diseases as described above. Determination of the association between APOE and the risk of pathogenesis is a considerable challenge, but it is essential for diagnosis, risk assessment, prevention, and treatment of disease in humans.

Acknowledgment

This work was supported by the grants from Gachon University Gil Medical Center (grant number 2013-22) and the Korea Health Technology R&D Project (HI14C3331) through the Korea Health Industry Development Institute (KHIDI), Korea Ministry of Health & Welfare.

Disclosure

The authors report no conflicts of interest in this work.

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